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Original Research Article

Fatty acids analysis of aerial parts of *Phyllanthus fraternus* Webster by gas chromatography-mass spectrometry

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ABSTRACT

Background: *Phyllanthus fraternus* Webster (Euphorbiaceae) is potentially used to treat diabetes, jaundice, dysentery, dyspepsia, indigestion, influenza, urinary tract diseases, kidney stones, skin eruptions and vaginitis in traditional systems of medicine. The objective of this study was to describe the systematic fatty acid composition of the petroleum ether extract of *P. fraternus* aerial parts.
Material and Methods: The chemical composition of a petroleum ether extract of the *P. fraternus* aerial parts was analyzed by gas chromatography-mass spectrometry technique.
Results: The extract was constituted of forty two compounds including saturated (31.14%) and unsaturated fatty (29.28%) acids, a cyclic fatty acid (0.41%), long chain aldehyde (11.36%), alcohols (6.82%) and fatty alcohols (5.37%), alkane (5.35%) and alkene (4.61%) hydrocarbons along with a lactone (1.03%), a steroid (2.27%), an alkylated phenol (0.27%) and a bicyclic sesquiterpene (1.02%).
Conclusion: Most of the fatty acids were identified as methyl esters. *P. fraternus* aerial parts have the potential to be exploited as a source of saturated and mono and polyunsaturated fatty acids.

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INTRODUCTION

Phyllanthus fraternus Webster (Euphorbiaceae) is a small erect annual herb growing up to 30-60 cm in height and is indigenous to the tropical areas of south-eastern Asia, western Pakistan, India, China and Amazon rainforest (Girach et al, 1994; Anonymous, 2005). Traditionally, the plant is used to treat kidney stones, urinary tract diseases, jaundice, dyspepsia, indigestion, diabetes, influenza, vaginitis, and skin eruptions. It possesses anti-inflammatory, diuretic, deobstruent, cholagogue, febrifuge, stomachic, astringent and styptic properties. An infusion of the plant is drunk as a blood purifier, to reduce blood sugar level and to treat dysentery; and a plant decoction with honey is taken to subside cough. It is ingested by mothers after childbirth to alleviate painful womb. The plant is added in the Ayurvedic herbal formulations such as Chitraka haritaki, Cyavanaprasa, Madhuyastyadi taila, Pippalyadi ghrita and Satavari guda (Anonymous, 2009). The plant is effective to relieve hepatotoxicity, hyperglycemia, fibromyalgia, pain, hepatitis B, malaria and viral and bacterial infections (Chopade & Sayyad, 2014; Chopra et al, 1986). n-Octacos-17-enoic acid, n-dodecanoyl-O-β-D-glucopyranosyl-(2′→1′′)-O-β-D-glucopyranosyl-(2′′→1′′′)-O-β-D-glucopyranosyl-(2′′′→1′′′′)-O-β-D-glucopyrano-side, 1-pentacosanol, β-sitosteryl oleate and linoleate,

stigmasterol, palmityl glucuronoside (Ali et al, 2015), alkamides (Sittie et al, 1998), seco-sterols, oxygenated heterocyclic compounds, diterpene ester and tetraterpene ketone have been reported from the plant (Gupta and Ali, 1999a; Gupta and Ali, 1999b; Gupta and Ali, 2000). Its uses as an important ingredient in various Ayurvedic formulations has encouraged us to establish a systematic fatty acid composition of the petroleum ether extract of *P. fraternus* aerial parts.

MATERIAL AND METHODS

Plant materials

The fresh aerial parts of *P. fraternus* were collected from the herbal garden of Jamia Hamdard, New Delhi. The plant sample was authenticated by Dr. H.B. Singh, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen of the plant sample was deposited in the herbarium of NISCAIR.

Preparation of extract

Finely grounded powder (100 g) of *P. fraternus* aerial parts was soaked in petroleum ether (3 x 250 ml) for eight hours and then filtered through a Whatmann filter paper No.1. Sodium sulphate was used to remove traces of moisture from the filtrates. The filtrates were concentrated under reduced pressure using a rotary evaporator at 45° C to get a yellowish white mass. The percentage yield of the petroleum ether extract was 2.27% w/w.

Fatty acid methyl ester (FAME) preparation

A one-step extraction-methylation procedure of Browse et al (1986) was applied with a slight modification to the petroleum ether extract. The extract (1 g) was mixed with 3 ml of methanolic sulphuric acid which was prepared by diluting a 3 M solution of sulphuric acid to 1 M of methanol. After cooling, 0.3 ml of hexane and 1 ml of 0.9% sodium chloride were added and the fatty acid methyl esters (FAMEs) were extracted by shaking. The sample was then centrifuged (1000 × g × 30 s) and the hexane layer was used for the fatty acid analysis.

GC-MS analysis

The analysis of the petroleum ether extract was carried out on a GC-MS system (Shimadzu QP-2010) with AB-Innowax 7031428 WCOT column (60 m x 0.25 mm x 0.25 μm). Helium was used as a carrier gas with a flow rate of 1.21 mL/min. The temperature of the oven was 80 ºC for 1 min and subsequently controlled isothermally for 2 min. Injector port: 270 ºC, detector: 280 ºC, split ratio 1:50, injected a volume of the sample: 1 μL. The recording was executed at 70 eV, scan duration 1.5 s; mass range 40-750 amu. Software implemented to handle mass spectra and chromatograph was a Chem station (Figure 1).

Identification of components

All the constituents were identified by comparison of their retention indices (RI) either with those of standard compounds available in author's library or with those of literature in close agreement to RI (Babushok et al., 2011; Ali, 2001; McLaerty, 1989). Further identification of components was carried out by comparison of mass spectra and their fragmentation patterns obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K.L, WILEY8 libraries, and published literature. Retention indices of the components were determined relative to the retention times of a series of n-alkanes relative to C9-C20 on HPS and HP-20M columns.

RESULT AND DISCUSSION

The study of the petroleum ether extract of the aerial parts of *P. fraternus* by GC-MS exhibited the presence of 42 compounds (Table 1). It mainly constituted of saturated (31.14%) and unsaturated fatty acids (29.28%), cyclic fatty acid (0.41%), long chain aldehyde (11.36%), alcohols (6.82%), fatty alcohols (5.37%), alkane (5.35%) and alkene (4.61%) hydrocarbons along with a lactone (1.03%), a steroid (2.27%), an alkylated phenol (0.27%) and a bicyclic sesquiterpene (1.02%). Five unsaturated and twelve saturated fatty acids were identified positively and a cyclic fatty acid, 2-[[2-[(2-ethylcyclopropyl) methyl cyclopropane octanoic acid was determined partially. All saturated and unsaturated fatty acids were analyzed as to their respective methyl esters. Saturated fatty acids were present in the highest percentage (31.14%) in *P. fraternus* petroleum ether extract. These fatty acids, all were positively characterized, included palmitic acid (14.69%), docosanoic acid (4.54%), octadecanoic acid (2.93%), tetracosanoic acid (2.51%), margaric acid (1.74%), eicosanoic acid (1.71%), pentacosanoic acid (0.90%), tetradecanoic acid (0.66%) with minor percentage of dodecanoic acid (0.44%), tricosanoic acid (0.30%) and pentadecanoic acid (0.16%). Among five unsaturated fatty acids, the major one was linolenic acid (20.47%) followed by linoleic acid (6.26%), epoxystearic acid (1.85%), linoleoyl chloride (0.42%) and 13-docosenoic acid (0.28%) (Table 1).

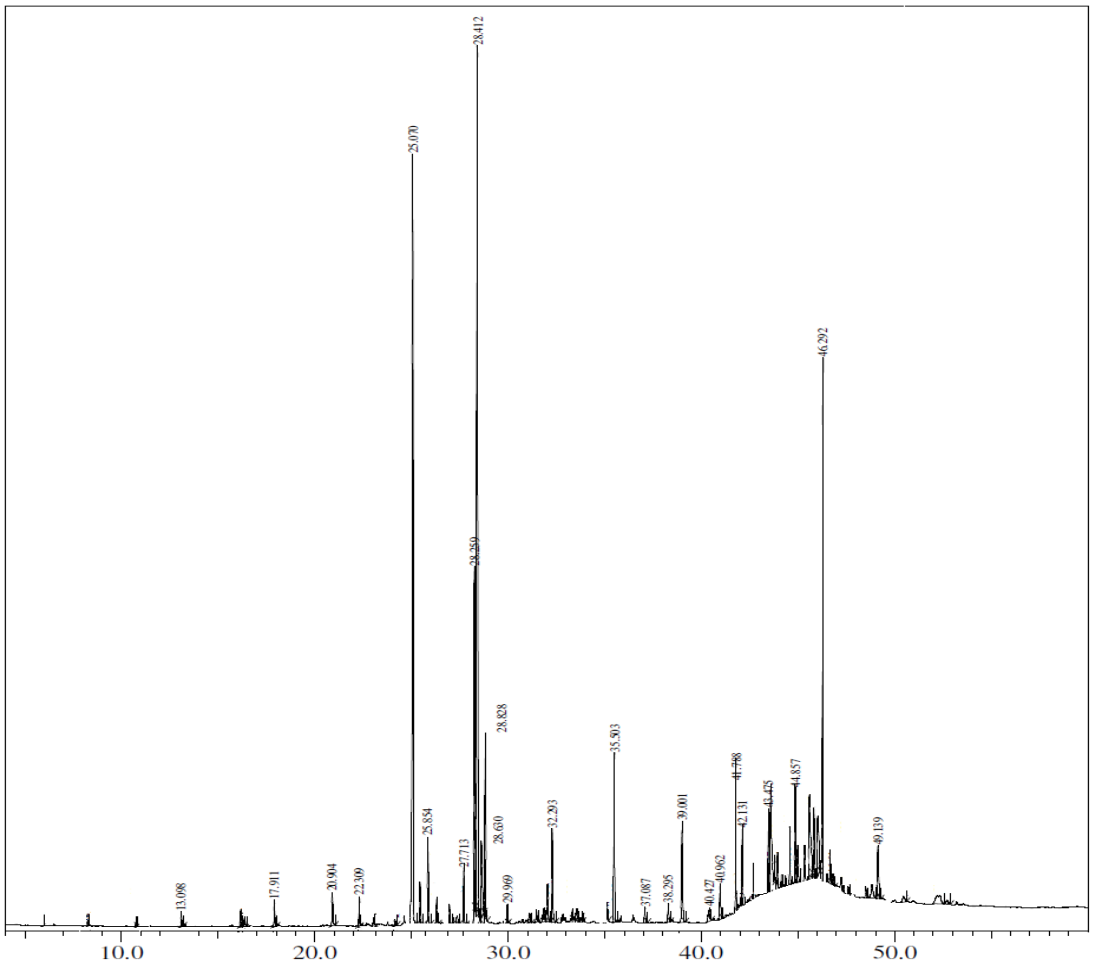

Figure 1. GC-MS spectrum of petroleum ether extract of aerial parts of *P. fraternus*.

Several types of alcohols characterized in small quantity were cyclic alcohol namely 1-ethyl-4,4-dimethyl-2-cyclohexen-1-ol (0.38%), two unsaturated aliphatic alcohols viz. 2,3-dimethyl-1-undecen-3-ol (1.50%) and 1,3,12-nonadecatriene-5,14-diol (0.39%), a terpenic alcohol solanesol (1.52%) and two acyclic diterpene alcohols phytol (2.19%) and isophytol (0.84%). Two fatty alcohols, 1-triacontanol (5.11%) and 1-eicosanol (0.26%) were also characterized under the group of alcohols. The petroleum ether extract was found to contain alkanes identified as tetratetracontane (2.53%), hexacosane (1.70%) and docosane (0.79%) and alkene hydrocarbons characterized as 1-nonadecene (1.50%), 2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene (1.18%), 2,6,10-trimethyl,14-ethylene-14-pentadecne (0.46%), 1-pentadecene (0.41%), tridecane (0.33%), 3-hexadecene (0.23%) together with a polyunsaturated triterpenic hydrocarbon, squalene (0.83%). A lactone (γ-undecalactone, 1.03%), bicyclic sesquiterpene caryophyllene oxide (1.02%), a steroid, γ-sitosterol (2.27%), three long chain aldehydes lauraldehyde dimethyl acetal (8.23%), stearyl aldehyde (1.83%) and olealdehyde dimethyl acetal (1.30%) and an alkylated phenol, 2,4-bis(1,1-dimethylethyl)-phenol (0.27%) were present in the extract (Table 1). However, esters of long chain fatty acids (85%), free fatty acids and hydrocarbons have already been reported (Anonymous, 2005). The seed oil of P. niruri contained ricinoleic acid (1.2%), and the other major components of the oil were linolenic acid (51.4%), linoleic acid (21%), palmitic acid (13.9%), stearic acid (5.3%) and oleic acid (7.2%). Linolenic acid represented 35% of the total fatty acid mixture and, therefore, this oil had been classified as linolenic-rich oils (Ahmad et al., 1981).

cis-Linoleic and α-linolenic acids are essential fatty acids for humans easily available in foods. Earlier reports exhibited that they are metabolized to their respective long-chain metabolites such as dihomo-γ-linolenic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid. They may act as HMG-CoA reductase inhibitors and endogenous angiotensin converting enzyme, anti-hypertensives, nitric oxide enhancers, and anti-atherosclerotic. They react with nitric oxide (NO) to give respective derivatives of nitroalkene that have cell-signaling actions. In various diseases such as hypertension, coronary heart disease, obesity, diabetes mellitus, alcoholism, Alzheimer's disease, atherosclerosis, schizophrenia and cancer, the metabolism of these essential fatty acids is altered.

Table 1. Chemical composition of petroleum ether extract of aerial parts of *P. fraternus*.

S. No.	Components	RI	Molecular formula	Molecular weight	RC
1.	Tridecane	1313	$C_{13}H_{28}$	184	0.33
2.	1-Ethyl-4,4-dimethyl-2-cyclohexen-1-ol	1328	$C_{10}H_{18}O$	154	0.38
3.	2,3-Dimethyl-1-undecen-3-ol	1372	$C_{13}H_{26}O$	198	1.50
4.	1-Pentadecene	1502	$C_{15}H_{30}$	210	0.41
5.	Caryophyllene oxide	1507	$C_{15}H_{24}O$	220	1.02
6.	Lauraldehyde dimethyl acetal	1513	$C_{14}H_{30}O_2$	230	8.23
7.	Phenol, 2,4-bis(1,1-dimethylethyl)-	1555	$C_{14}H_{22}O$	206	0.27
8.	Dodecanoic acid methyl ester	1571	$C_{13}H_{26}O_2$	214	0.44
9.	3-Hexadecene, (Z)-	1620	$C_{16}H_{32}$	224	0.23
10.	Tetradecanoic acid methyl ester	1680	$C_{15}H_{30}O_2$	242	0.66
11.	Pentadecanoic acid methyl ester	1779	$C_{16}H_{32}O_2$	256	0.16
12.	Isophytol	1899	$C_{20}H_{40}O$	296	0.84
13.	1-Nonadecene	1908	$C_{19}H_{38}$	266	1.50
14.	Palmitic acid methyl ester	1958	$C_{17}H_{34}O_2$	270	14.69
15.	Margaric acid methyl ester	1978	$C_{18}H_{36}O_2$	284	1.74
16.	Stearyl aldehyde	1999	$C_{18}H_{36}O$	268	1.83
17.	Phytol	2045	$C_{20}H_{40}O$	296	2.19
18.	2,6,10-Trimethyl,14-ethylene-14-pentadecne	2048	$C_{20}H_{38}$	278	0.46
19.	Octadecanoic acid methyl ester	2077	$C_{19}H_{38}O_2$	298	2.93
20.	Linoleic acid methyl ester	2093	$C_{19}H_{34}O_2$	294	6.26
21.	Linolenic acid methyl ester	2101	$C_{19}H_{32}O_2$	292	20.47
22.	Olealdehyde dimethyl acetal	2105	$C_{20}H_{40}O_2$	312	1.30
23.	Solanesol	2138	$C_{45}H_{74}O$	630	1.52
24.	Linoleoylchoride	2139	$C_{18}H_{31}ClO$	298	0.42
25.	Docosane	2208	$C_{22}H_{46}$	310	0.79
26.	Epoxysteric acid methyl ester	2219	$C_{18}H_{34}O_3$	298	1.85
27.	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	2241	$C_{19}H_{34}O_2$	294	0.39
28.	2-[[2-[(2-Ethylcyclopropyl) methyl cyclopropaneoctanoic acid	2266	$C_{22}H_{38}O_2$	334	0.41
29.	γ-Undecalactone	2270	$C_{11}H_{20}O_2$	184	1.03
30.	1-Eicosanol	2293	$C_{20}H_{42}O$	298	0.26
31.	Eicosanoic acid methyl ester	2298	$C_{21}H_{42}O_2$	326	1.71
32.	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	2432	$C_{25}H_{42}$	342	1.18
33.	Docosanoic acid methyl ester	2475	$C_{23}H_{46}O_2$	354	4.54
34.	13-Docosenoic acid methyl ester	2483	$C_{23}H_{44}O_2$	352	0.28
35.	Tricosanoic acid methyl ester	2574	$C_{24}H_{48}O_2$	368	0.30
36.	Hexacosane	2674	$C_{26}H_{54}$	366	1.70
37.	Tetracosanoic acid methyl ester	2730	$C_{25}H_{50}O_2$	382	2.51
38.	Pentacosanoic acid methyl ester	2773	$C_{26}H_{52}O_2$	396	0.90
39.	Squalene	2914	$C_{30}H_{50}$	410	0.83
40.	1-Triacontanol	3246	$C_{30}H_{62}O$	438	5.11
41.	γ-Sitosterol	3414	$C_{29}H_{50}O$	2731	2.27
42.	Tetratetracontane	4395	$C_{44}H_{90}$	618	2.53

Thus, cis-linoleic and α-linolenic acids and their derivatives have noteworthy clinical consequences (Das, 2006a; Das, 2006b). The high consumptions of linoleic acid protect from the cancer progression, possibly through the generation of 13-hydroxyoctadecadienoic acid (Horrobin and Ziboh (1997). However, among five unsaturated fatty acids, the petroleum ether extract of the aerial parts contained the highest percentage of linolenic (20.47%) and linoleic acids (6.26%).

CONCLUSION

The present study has enhanced the phytochemical nature and fatty acid composition of the aerial parts of *P. fraternus*. The petroleum ether extract of *P. fraternus* aerial parts is rich in saturated and unsaturated fatty acids. However, the major unsaturated fatty acids were linolenic (20.47%) and linoleic acid (6.26%) which protected against the cancer development and acted as cardioprotective, anti-atherosclerotic and nitric oxide enhancers. In conclusion, *P. fraternus* aerial parts have

the potential to be exploited as a source of saturated and mono and polyunsaturated fatty acids.

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CONFLICT OF INTEREST

None declared.

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